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Fusarium commune is a new species identified by morphological and molecular phylogenetic data

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Abstract: *Fusarium commune* sp. nov. was isolated from soil and *Pisum sativum* in Denmark and several widespread locations within the northern hemisphere from diverse substrates including white pine, Douglas fir, carnation, corn, carrot, barley and soil. *Fusarium commune* is characterized by and distinguished from its putative sister taxon, the *F. oxysporum* complex, in having long, slender monophialides and polyphialides when cultured in the dark. Based on the combined DNA sequence data from translation elongation factor 1α (EF-1α) and the mitochondrial small subunit ribosomal DNA (mtSSU rDNA), the 15 isolates of *F. commune* analyzed formed a strongly supported clade closely related to but independent of the *F. oxysporum* and *Gibberella fujikuroi* species complexes.

Key words: gene genealogies, hyphomycetes, mitochondrial small subunit rDNA, phylogeny, soil fungi, translation elongation factor α

INTRODUCTION

During a study of *Fusarium oxysporum* Schlect. emend. Snyder & Hansen occurring in pea fields in Denmark, several fusaria were isolated that could not be identified as any described species (Gerlach and Nirenberg 1982, Booth 1971, Nelson et al 1983, Nirenberg and O'Donnell 1998). These cultures shared several morphological characteristics typically found in *F. oxysporum*, but they differed in that they produced polyphialides as well as long, slender monophialides.

Morphological characters frequently are homoplastic, and the circumscription of taxa, based on the size and shape of conidia and conidiophores and the color and texture of colonies, has resulted in an underestimation of species diversity within *Fusarium* Link (Brayford 1996, O'Donnell 1996). Phylogenetic species recognition, based on DNA sequence data from multiple loci, allows greater numbers of species to be distinguished than in the exclusive use of morphological features (Taylor et al 2000). Based on morphological characters alone, between two and 10 taxa have been recognized in section *Liseola* (Booth 1971, Gerlach and Nirenberg 1982, Nelson et al 1983), four species in section *Dlaminia* (Kwasna et al 1991) and two species in section *Elegans* (Gerlach and Nirenberg 1982). Using multigene genealogies, O'Donnell et al (1998) recognized 36 species within the *Gibberella fujikuroi* Saw. complex, represented by part or all species of sections *Liseola*, *Elegans* and *Dlaminia*. Over the past half-decade, combined molecular phylogenetic and morphological approaches have been shown to be invaluable in the diagnosis of fusaria (Aoki and O'Donnell 1999, Aoki et al 2001, Gams et al 1999, Geiser et al 2001, Nirenberg and O'Donnell 1998, O'Donnell et al 1998).

Evolutionary relationships among and within the *F. oxysporum* species complex have been investigated with multilocus DNA sequence data (Baayen et al 2000, Skovgaard et al 2001). *Fusarium hostae* Geiser, a species causing root and crown rot of hosta, recently was discovered as the putative sister taxon of *F. redolens* Wollenw., based on the analysis of partial β-tubulin and translation elongation factor 1α sequences (Baayen et al 2001, Geiser et al 2001). In this study we describe a new *Fusarium* species based on morphology and phylogenetic analysis of partial (EF-1α) and the mitochondrial small subunit ribosomal DNA (mtSSU rDNA) sequences.

MATERIALS AND METHODS

Strains used in this study are listed in TABLE I together with substrate and geographic origin. All isolates are stored in

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TABLE I. Strains of *Fusarium* used in this study

Species	Source[a]	Geographic origin	Host/Substrate
F. beomiforme	NRRL 25174 (FRC M-1425 = BBA 65829)	New Caledonia	Soil
F. commune	NRRL 22900 (BCRI P4C2P17A)	B.C., Canada	*Pseudotsuga menziesii* (Mirb.) Frane
F. commune	NRRL 22903[b] (BCRI 3139)	OR, USA	*Pseudotsuga menziesii*
F. commune	NRRL 25043 (BBA 69585)	Ontario, Canada	*Pinus strobes* L.
F. commune	NRRL 25049 (BBA 69586)	Ontario, Canada	*Pinus strobes*
F. commune	NRRL 26897 (ARCF 93144)	Finland	Barley root (*Hordeum vulgare* L.)
F. commune	NRRL 26898[d] (ARCF 94193 = BBA 71641)	Finland	Carrot root (*Daucus carota* L.)
F. commune	NRRL 28058 (FRC 0-1173)	Japan	River sediment
F. commune	NRRL 28180 (MA 1208)	Austria	*Zea mays* L. leaf
F. commune	NRRL 28182 (MA 1210)	Austria	*Zea mays* leaf
F. commune	NRRL 28387[d] (PD 90/1377)	The Netherlands	*Dianthus caryophyllus* L.
F. commune	NRRL 31076[bcd] (AAS 156 = BBA 71639)	Denmark	*Pisum sativum* L.
F. commune	NRRL 31077[d] (AAS 345)	Denmark	Soil
F. commune	NRRL 31079[d] (AAS 362)	Denmark	Soil
F. commune	NRRL 31080 (AAS 363 = BBA 71640)	Denmark	Soil
F. commune	NRRL 31081 (AAS 364)	Denmark	Soil
F. hostae	NRRL 29889 (FRC 0-2074)	SC, USA	*Hosta* sp.
F. oxysporum	NRRL 25603 (HCK A2)	Australia	*Musa* sp.
F. oxysporum	NRRL 31073 (Bødker L5)	Sweden	*Pisum sativum*
F. oxysporum	NRRL 31074 (AAS 112)	Denmark	*Pisum sativum*
F. oxysporum	NRRL 31078 (AAS 350)	Denmark	Soil
F. proliferatum (Matsushima) Nirenb.	NRRL 22057 (JFL 4853)	—	—
F. redolens	NRRL 31075 (AAS 127)	Denmark	*Pisum sativum*
F. redolens	NRRL 31255 (AAS 120)	Denmark	*Pisum sativum*
F. subglutinans (Wollenw. & Reinking) Nelson et al.	NRRL 22016 (JFL 2192)	USA	*Zea mays*
F. verticillioides (Sacc.) Nirenb.	NRRL 22172 (BBA 62264)	Germany	*Zea mays*

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[b] Pathogenicity tested at BBA.

[c] Ex-type.

[d] Isolates examined.

liquid nitrogen at the National Center for Agricultural Utilization Research, Peoria, IL (NRRL). The ex-type culture of *Fusarium commune* NRRL 31076 also is stored in soil vials and as freeze-dried cultures at the Institut für Pflanzenvirologie, Mikrobiologie und biologische Sicherheit, Biologische Bundesanstalt für Land- und Forstwirtschaft culture collection (BBA), Berlin, Germany, as well as in glycerol at −80 C at the Botanical Institute, Department of Mycology, University of Copenhagen, Copenhagen, Denmark (AAS).

Morphological examination.—Cultures were grown on potato-dextrose agar (PDA; Difco, Detroit, Michigan) at 20 C in the dark. Colony colors were determined using the Methuen Handbook of Colour (Kornerup and Wanscher 1978). Microscopic characters were studied after 10 to 14 days' growth on synthetic low nutrient agar (SNA) overlain with a 1 × 2 cm piece of sterile filter paper (Nirenberg 1990). Characteristic morphological traits were photographed and measurements of conidia, chlamydospores and phialides were made after the cultures were incubated either in the dark or under continuous black light (Philips TLD 18w/08) (Nirenberg 1990). At least 30 randomly selected 1-, 3- and 5-septate conidia were measured and their mean values and ranges (shown in brackets) were determined.

Pathogenicity test.—Two isolates of *Fusarium commune*, NRRL 22903 and 31076, were tested for their pathogenicity toward *Pinus sylvestris* L. and *Picea rubens* Sarg. These conifer species are hosts of *F. blasticola* Rostr., a morphologically similar species initially thought to be conspecific with *F. commune* (see below). From 14-day-old SNA cultures of these two isolates, five 10 mm² pieces were transferred separately to each of seven Erlenmeyer flasks filled with a sterile peat-straw-sand mixture (2:2:1). Each culture was allowed to grow through the mixture at 20 C. After 19 d the inoculated soil was mixed with a commercial soil, TKS 1 (Flora Gard, Berlin, Germany), and sand (2:3:1). The inoculum of each fungus was used to fill 20 plastic pots (9 cm in diameter). Eight-week-old seedlings of each of the conifer species were planted individually in 10 pots. Negative controls treated the same way but lacked fungi. The pots were placed in a greenhouse at 15 C to 18 C. After three months the temperature was raised to 25 C for the next two months. Symptoms were evaluated after five months' incubation.

Sequencing and phylogenetic analysis.—Isolates were grown as shake cultures (200 rpm) in a yeast-malt broth (0.3% yeast extract, 0.3% malt extract, 0.5% peptone, 2% glucose) 2–3 days at room temperature. Genomic DNA was extracted from lyophilized mycelium by the CTAB-method (O'Donnell and Cigelnik 1997). Amplification and sequencing of the mtSSU rDNA and EF-1α genes was carried out employing the primers and thermocycling parameters described by White et al (1990) and O'Donnell et al (2000). PCR products were purified with GeneClean II (Bio 101, La Jolla, CA). Cycle sequencing products were spun through Sephadex G-50 columns (Pharmacia, Piscataway, NJ) to remove unincorporated dye-labeled nucleotides and sequenced on an automated ABI 377 sequencer (Perkin-Elmer, Foster City, CA).

Sequencher 3.0 (GeneCodes, Ann Arbor, MI) and Bioedit (Hall 1999) were used to edit and align the sequence data. The final alignment is available through TreeBASE. Sequences were deposited in GenBank under accessions numbers AF362261 to AF362292. Sequence data from EF-1α and mtSSU rDNA were tested for combinability with the partition homogeneity test implemented in PAUP 4.0b2 (Swofford 1999). Branch and bound searches were performed with default options. Alignment gaps were treated as missing data and 1000 parsimony bootstrap replications were conducted to test clade support.

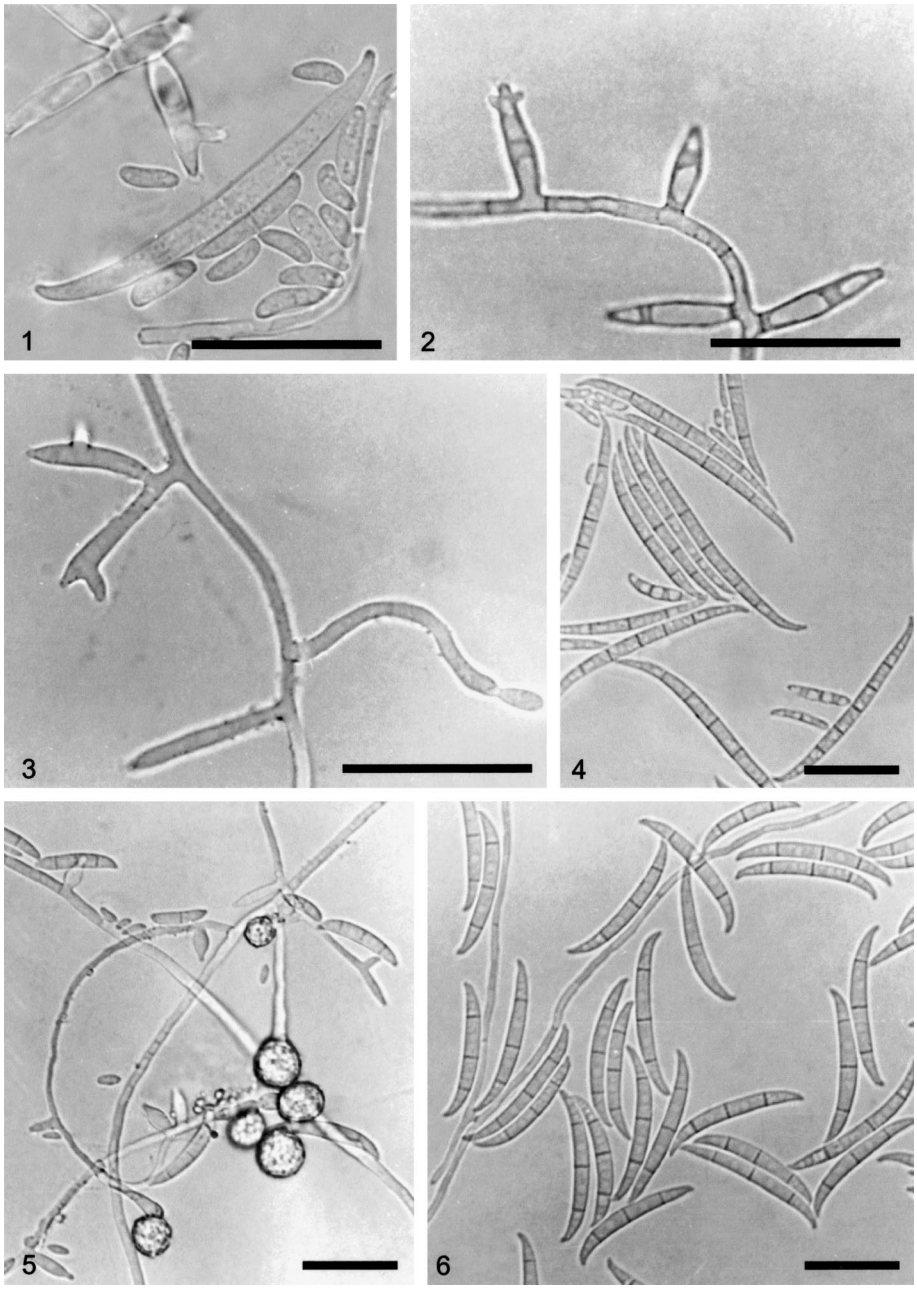
RESULTS

Fusarium commune Skovgaard, O'Donnell et Nirenberg, sp. nov. FIGS. 1–6

Coloniae in PDA in dies radium 4.6–5.6 mm expandentes, temperatura 20 C in obscuritate. Mycelium aerium album usque ad aurantiaco-album, lanosum usque ad byssaceum. Color coloniarum in reverso griseo-fulvidus, nonnumquam griseo-violaceus. Odor insensibilis. Sporulatio in mycelio aerio praecox in SNA, conidia capitulis aggregata. Sporodochia post 10 dies formata sub luce nigra continua. Conidiophora in mycelio aerio prostrata. Phialides cylindricae pro parte maxima monophialidicae nonnumquam polyphialidicae; monophialides seu breves (ca 17 μm) seu longiores (ad 60 μm), 3.5–4.0 μm latae; polyphialides not magis quam 30 μm longae. Conidia in mycelio aerio oblonge ovalia, recta vel curvata, plerumque non-septata, aliquando 1- vel 2-septata, conidia non-septata: (4.0–) 5.5–7.7 (-8.0) × (2.0–) 2.5–3.5 (–4.0) μm; conidia sporodochialia plerumque falcata et 3-septata: (22–) 24–30 (–38) × 3.8–4.1 μm obscuritate, (32–) 34–42 (–50) × 3.8–4.2 μm sub luce nigra continua; chlamydosporae 8–12 μm diam, leves, singulares vel binae. Teleomorphosis ignota. Holotypi origo geographica in Dania, in humo. Ex holotypo culturae NRRL 31076 = BBA 71639 = AAS 156.

HOLOTYPUS. Colonia sicca BBA 71639, deposita in herb. B.

Colonies with a radial growth rate of 5.1 mm per day on PDA at 20 C in the dark. Aerial mycelium white to orange white, generally abundant, densely floccose to fluffy, later resupinate in degenerated cultures. Colony reverse grayish yellow with magenta to dull violet pigmentation, often in rings. The grayish magenta was dominant in older and degenerate cultures. Odor not detectable. Sporulation starting early in aerial mycelium, abundant after 10 days on SNA. 0-septate conidia produced in slimy droplets and sporodochia, typically formed after 10 days under continuous black light. Conidiophores consisting of short monophialides up to 17 μm long and 4.0 μm wide, or longer and more slender monophialides up



FIGS. 1–6. *Fusarium commune* (Scale bar = 25 μm). 1. Polyphialidic conidiophore. 2. Polyphialidic and monophialidic conidiophores of the aerial mycelium. 3. Polyphialidic and long monophialidic conidiophores of the aerial mycelium. 4. Long sporodochial conidia. 5. Chlamydospores. 6. Sporodochial conidia. Figs. 1–3, 5 from cultures maintained in the dark, Figs. 4, 6 from cultures maintained under continuous black light.

to 60 μm long and 3.5 μm wide. Polyphialides up to 30 μm long and 3.5 μm wide, appearing in cultures incubated in the dark. Aerial conidia mostly 0-septate, cylindrical, straight to slightly curved, measuring (4.0–) 5.5–7.7 (–8.0) × (2.0–) 2.5–3.5 (–4.0) μm. Conidia borne in sporodochia typically fusiform with a slightly curved apical cell and a foot-shaped basal cell, bending equally toward both ends. Three-septate conidia (22–) 24–30 (–38) × 3.8–4.1 μm formed in the dark and under continuous black light (32–) 34–42 (–50) × 3.8–4.2 μm, five-septate conidia under continuous black light (52–) 56–60 (–64) × (3.8–) 3.9–4.6 (–5.0) μm. Chlamydospores 8–12 μm diam, smooth, intercalary or terminal, and produced singly or in pairs. Cardinal temperatures: 7.5 C, 27.5 C and 35.0 C. Teleomorph unknown.

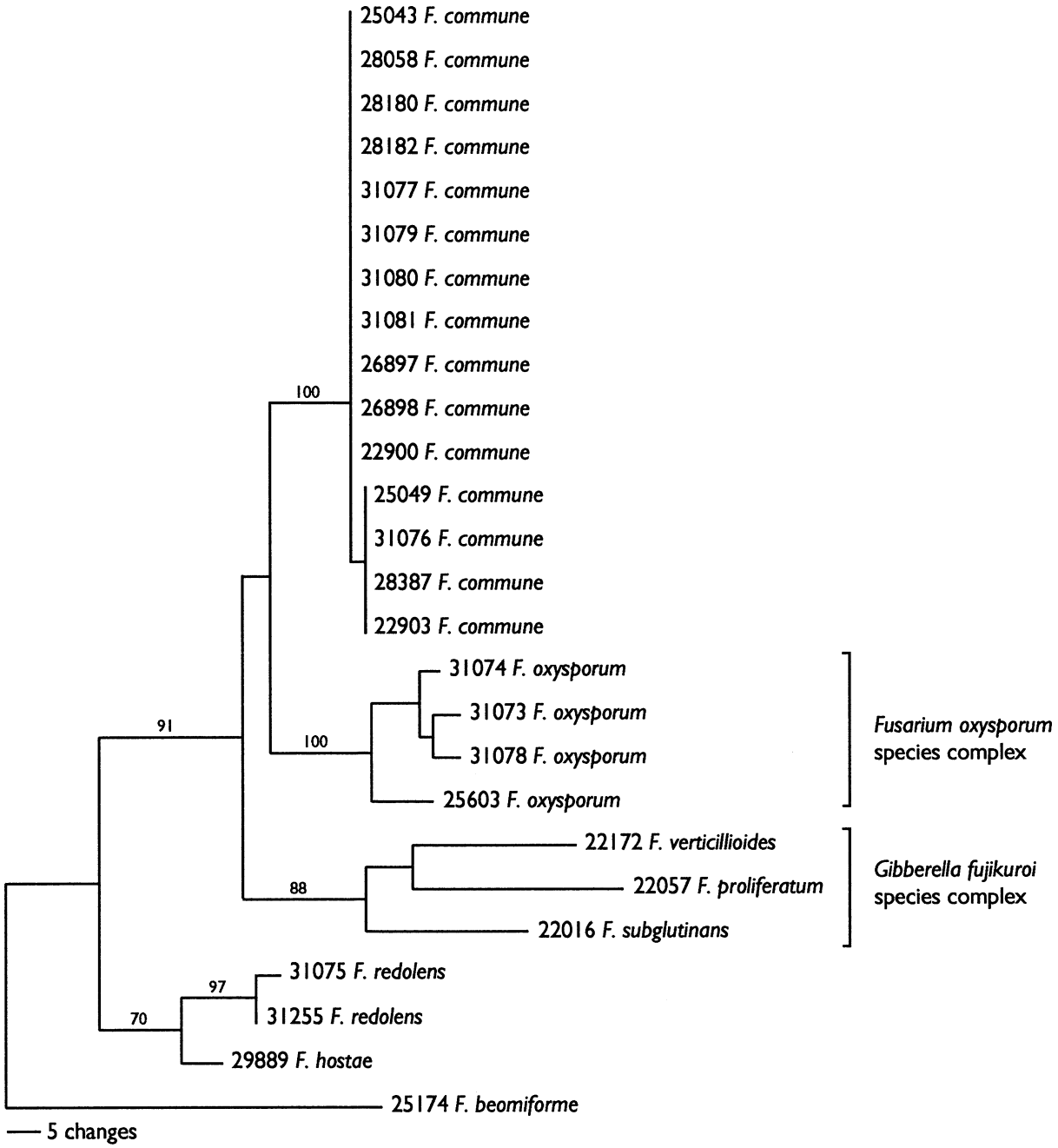


FIG. 7. Single most-parsimonious phylogram based on the combined analysis of EF-1α and mtSSU rDNA gene sequences. Bootstrap frequencies from 1000 replications are given above nodes. Consistency index = 0.80, retention index = 0.89, tree length = 285.ol6

Sequence data.—Two substitutions were found within the alignment of EF-1α (572 bp) and mtSSU rDNA (698 bp) from the 15 isolates of *F. commune*. Results of the partition homogeneity test indicated that the EF-1α and mtSSU rDNA partitions could be combined (P = 0.267). A branch and bound search of the combined dataset, rooted with sequences of *F. beomiforme* Nelson et al, yielded a single most-parsimonious tree of 285 steps (FIG. 7, consistency index = 0.80, retention index = 0.89). The 15 isolates of *F. commune* formed a strongly supported monophyletic group (bootstrap = 100%).

Pathogenicity test.—Disease symptoms were not observed after five months incubation of *Pinus sylvestris*

and *Picea rubens* with strains of *F. commune* (NRRL 22903 and 31076).

DISCUSSION

Fusarium commune and *F. oxysporum* are morphologically similar in that they both produce conidia on short monophialides in false heads on the aerial mycelium and chlamydospores singly or in pairs. Unique features of *F. commune* include long, slender monophialides in addition to the occasional production of polyphialides. Ten of the 15 strains of *F. commune* used in this study were identified originally as *F. oxysporum*.

Four of the 15 isolates studied (NRRL 22900, 25043, 28058, 31080) produced longer sporodochial conidia than the type isolate, and the other isolates in the dark and under black light. In these four strains, 3-septate conidia measured (36–) 40–50 (–52) × 3.7–4.0 μm in the dark and (36–) 44–56 (–60) × 3.8–4.1 μm under continuous black light; 5-septate conidia were (44–) 52–58 (–60) × 3.9–4.1 μm under continuous black light.

Little variation in EF-1α and mtSSU rDNA sequences was observed within *F. commune*, even though the 15 strains were isolated from a wide range of substrates and geographic locations throughout the northern hemisphere. No relationship was observed between the minor sequence differences in the *F. commune* clade and differences in macroconidial morphology. The molecular phylogenetic analysis identified *F. commune* as a putative sister group to the *F. oxysporum* complex, a result consistent with the high morphological similarity of these taxa (FIG. 7). *Fusarium redolens* as well as the newly described *F. hostae* (Geiser et al 2001) formed a sister group to the rest of the ingroup taxa.

Fusarium commune was considered to be conspecific with *F. blasticola* (Wollenweber and Reinking 1935), a species isolated originally from seedlings of *Pinus montana* Lamarck and described as *Fusoma parasitica* Tub. (Tubeuf 1895). Although conidia were not observed in the type material of *F. blasticola*, the description of this species as possessing slender (3.5 μm) 3-septate conidia that occur rarely in sporodochia (Hartig 1892, Wollenweber and Reinking 1935) is inconsistent with our observations of *F. commune*. In addition, results of the infection tests indicate that *F. commune* is not pathogenic to seedlings of *Pinus sylvestris* and *Picea rubens*, two of the hosts of *F. blasticola*. Collectively, these results support the separation of *F. commune* and *F. blasticola*. The phytopathological role of *F. commune* on other hosts, if any, is still unknown. Given that a culture extract of *F. commune* NRRL 28058 was reported to be toxigenic (Ueno et al 1977, Marasas et al 1984), studies are in progress to elucidate the mycotoxin potential of *F. commune*.

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